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Acute Myeloid Leukemia

Low frequency of exon 3 *PTPN11* mutations in adult *de novo* acute myeloid leukemia. Analysis of a consecutive series of 173 patients

A total of 173 samples obtained from adult patients with *de novo* acute myeloid leukemia (AML) were assayed for exon 3 *PTPN11* mutations by single strand conformation polymorphism (SSCP) analysis and direct sequencing. Only three monocytic leukemias had point mutations (1.73%).

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The *PTPN11* gene encodes the non-receptor-type protein tyrosine phosphatase SHP-2 (src homology region 2-domain phosphatase-2) which plays an important role in the response to extracellular stimuli and is required for the activation of the RAS/MEK/ERK kinase pathway. *PTPN11* has been identified as the gene causing Noonan syndrome and it has also been found to be mutated in 34% of cases of juvenile myelomonocytic leukemia.[1,2] Reported acquired mutations in myeloid malignancies cause a gain-of-function of the SHP-2 protein.[3,4]

To assess the relevance of acquired *PTPN11* mutations in *de novo* adult AML we performed a mutational analysis in a consecutive series of 173 patients enrolled in the Spanish CETLAM protocol. DNA obtained from the diagnostic bone marrow was purified by digestion with proteinase K, extraction by the *salting out* method, and precipitation with ethanol. The configuration of the *MLL* locus was analyzed by hybridizing *BamHI*- and *Hind III*-digested DNA to the B859 probe. AML1-ETO and CBF,-MYH11 transcripts were assayed by reverse transcription polymerase chain reaction (RT-PCR) methods. The *FLT-3* tandem duplication of the *JM* domain was investigated by genomic PCR. The *D835* mutation was ruled out by means of DNA-PCR followed by EcoRV enzyme digestion. Detection of exon 3 *PTPN11* gene mutations was studied by means of radioactive SSCP. The primers and PCR protocols have been published elsewhere.[1,2] Direct sequencing was performed using forward and reverse primers in these cases with abnormal conformers on SSCP analysis using the ABI Prism dRhodamine Terminator Cycle Sequence Ready Reaction kit (PE Biosystems, Warrington, UK) and the ABI PRISM 310 Genetic Analyzer (Foster City, CA, USA).

Table 1. *PTPN11* mutated AML cases.

Pt.	Sex	Age	Dx	WBC	Cytogenetics	FLT3, MLL	PTPN11	Outcome
1	M	59	M5	73×10^9/L	45,XY,-7	GL	G60V	Dead 5m
2	F	48	M5	145×10^9/L	46,XX	GL	A72V	Dead 1m
3	M	35	M5	19×10^9/L	47,XY,+8	GL	H53Q	Dead 3m

M: male F: female; Dx: diagnosis according to FAB classification; GL: germ line.

We detected three M5 AML cases harboring point mutations (Table 1), representing a prevalence of 1.73%. These findings are in line with those reported by Johan *et al.*[5] in 64 AML cases enrolled in the MRC trials and suggest that this molecular lesion is rare in *de novo* adult AML, and clearly less common than in AML in children. Interestingly, most pediatric cases corresponded to monocytic leukemias. Mutations at codon 60 and 72, located at interaction sites between the N-SH2 and PTP domains, have been previously reported whereas the mutation at codon 53 has not been described to date. One of the cases reported here showed a +8, another a –7, and the remaining case had a normal karyotype. We were not able to detect the associated *FLT3* or *MLL* rearrangements commonly encountered in monocytic leukemias. All three patients died within a relatively short period: patient #1 from infectious complications arising in the context of graft-versus-host disease, patient #2 from severe bleeding in the induction phase and patient #3 relapsed. It can be concluded that *PTPN11* mutations affect a low percentage of patients with *de novo* AML and appear to be restricted to cases with a monocytic differentiation.

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Acute Myeloid Leukemia

Tetraploidy or near-tetraploidy clones with double 8;21 translocation: a non-random additional anomaly of acute myeloid leukemia with t(8;21)(q22;q22)

We report on 6 patients with tetraploidy or near-tetraploidy acute myeloid leukemia (AML) with double t(8;21)(q22;q22) and review the literature on cases with the same cytogenetic abnormalities. Some common features were revealed by this analysis.

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The cytogenetic abnormality of tetraploidy or near-tetraploidy is a rare finding in acute myeloid leukemia (AML). Lemez *et al*.[1] divided near-tetraploidy AML into 2 categories: primary and secondary according to its origin. It is known that patients with primary near-tetraploidy AML manifest some common features: (i) near-tetraploidy karyotypes in most of the bone marrow metaphases examined at diagnosis of AML; (ii) the presence of giant myeloid blasts and dysplastic morphology in erythroid and/or megakaryocytic lineages in the bone marrow, pointing to the origin from pluripotent myeloid progenitor cells; (iii) expression of CD34 antigen; (iv) low yields of granulocyte- macrophage colony-forming units (GM-CFU) in culture; (v) a preceding preleukemic phase before the onset of the disease, and (vi) a poor prognosis. So far secondary tetraploidy or near-tetralpoidy AML has been associated with multiple structural chromosomal aberrations. There have been seven cases of AML with clearly secondary tetraploidy or near-tetraploidy metaphases with duplication of t(8;21)(q22;q22) at diagnosis or during the course of leukemia reported in the literature[2-6] (Table 1). We report here another six such cases.

Between January 1990 and December 2003, 216 cases of t(8;21) (q22;q22) AML were diagnosed in our institute, and all of them were AML-M2 subtype according to the FAB criteria. The patients' ages ranged 3–65 years with a median of 28 years; 125 were male and 91 female. Seventy-three patients(33.8%) presented with coexistence of normal and abnormal karyotypes, an additional chromosome aberration occurred in 146/216 (67.6%) of the patients and 53 (24.5%) had a complex karyotype (≥3 chromosomal abnormalities). The most frequent additional abnormalities were loss of a sex chromosome (41.2%), partial deletion of the long arm of chromosome 9 (23 cases, 10.6%) and less often, deletion or partial deletion of the long arm of chromosome 7 (6 cases, 2.85%). Other additional structural anomalities included

Table 1. Clinical and genetic features of the 13 cases of tetraploidy or near-tetraploidy AML with double t(8;21)(q22;q22).

Case	*Sex/age(years)*	*Karyotype*	*Survival (months)*	*Reference*
1	M/28	90,XX,-Y,-Y, t(8;21)(q22;q22)?2[5]/46,XY,-1, t(8;21)(q22;q22), +der(1), t(1;?) (p36;?) [2] /46,XY[4]	23	Testa[2]
2	M/53	46,XY/90,XX,-Y?2,-11, t(8;21)(q22;q22)?2,+mar[92.5%]	12+	Abe[3]
3	M/31	46,XY, t(8;21)(q22;q22)[2]/46,XY,add(7)(q31) t(8;21)(q22;q22)[6]/ 92,XXYY, add(7) (q31)?2, t(8;21)(q22;q22)?2[70]/46,XY[10]	4	Xue[4]
4	F/8	46,XX, t(8;21)(q22;q22)[13]/ 46,XX,+4 t(8;21)(q22;q22)[45]/ 92,XXXX,+4?2, t(8;21)(q22;q22)?2[38]/46,XX[1]	6	Xue[4]
5	F/10	46,XX, t(8;21)(q22;q22)[6]/110-117,XXX,-X,-X,-1,+4,+4,-7,add(7)(q31)?3, t(8;21)(q22;q22)?2,+der(21) t(8;21)(q22;q22),+22[272] / 92,XXXX,add(7) (q31)?2, t(8;21)(q22;q22)?2[21]/46,XX[7]	8	Xue[5]
6	M/7	45,X,-Y, t(8;21)(q22;q22)[6]/90,XX,-Y,-Y, t(8;21)(q22;q22)?2[32]/46,XY[2]	20+	Xue[5]
7	F/61	45,X,-X, t(8;21)(q22;q22)[1]/90,XXX,-X, t(8;21)(q22;q22)?2,-9[7]/46, XX[12]	3	Yamamoto[6]
8	F/6	46,XX,t(8;21)(q22;q22)[13]/92,XXXX,t(8;21)?2[2]	12	Present case[1]
9	M/48	41-44,X,-Y,-15,-17,t(8;21)(q22;q22),9q+,11q-,+Mar[cp2]/92,xxyy,der(22) t(4;22) (q10;q10)?2,t(8;21)?2,9q+?2,-11,-20,+Mar[cp15]	9	Present case[2]
10	F/9	46,XX,t(8;21)(q22;q22)[7]/92,XXXX,t(8;21)?2[8]	11	Present case[3]
11	M/35	46,XY,t(8;21)(q22;q22),-10,+11[1]/45,X,-Y,t(8;21)(q22;q22)[3]/46,XY,t(8;21)[1] /92, XXYY, t(8;21)?2[4]/46,XY[7]	3	Present case[4]
12	F/11	46,XX, t(8;21)(q22;q22)[10]/88-96, XXXX, t(8;21)(q22;q22) ?2 [cp15]	4	Present case[5]
13	F/12	46,XX,t(8;21)(q22;q22)[5]/92,XXXX,t(8;21)(q22;q22) ?2 [7]/46,XX[2]	6	Present case[6]